

## Intracellular Bacteria find the “Right” Motion

**Edith Gouin,<sup>1,2,3</sup> Juan-Jose Quereda Torres,<sup>1,2,3</sup> and Pascale Cossart<sup>1,2,3,\*</sup>**

<sup>1</sup>Unité des Interactions bactéries-Cellules, Institut Pasteur, 75015 Paris, FRANCE

<sup>2</sup>Inserm U604, 75015 Paris, FRANCE

<sup>3</sup>INRA USC2020, 75015 Paris, France

\*Correspondance : [pcossart@pasteur.fr](mailto:pcossart@pasteur.fr)

Benanti *et al* report that *Burkholderia pseudomallei* and *Burkholderia mallei* bacteria express proteins that mimic Ena/Vasp family proteins to polymerize actin thereby inducing actin-based motility. Thus bacteria can use the various cellular actin polymerization mechanisms for intra- and inter-cellular dissemination.

Intracellular bacterial pathogens such as *Listeria monocytogenes* and *Shigella flexneri* received a lot of attention in the early nineties when it was discovered that, after internalization by mammalian cells and escape from the endocytic vacuole, they actively recruit monomeric actin and polymerize it into filaments. This actin assembly process creates a force that then propels the bacteria through the cytosol. In this issue of Cell, Benanti *et al.* (2015) reveal that bacterial pathogens *Burkholderia pseudomallei* and *Burkholderia mallei* move inside cells by mimicking one of the several cellular mechanisms that control actin polymerization.

The study of bacterial motility led to the discovery of the role of Arp2/3 in actin polymerization (Welch et al., 1998). This seven protein complex needs to be activated by WASP family proteins to generate a dense array of actin filaments. Polymerization of actin filaments takes place on preformed filaments at an angle of 70°, a characteristic feature that can be visualized in cells by electron microscopy or *in vitro* using fluorescent actin and Arp2/3. Other nucleators, such as formins or Ena/VASP proteins as more recently shown (Winkelman et al., 2013) generate linear long filaments. These proteins act processively on barbed ends in which they sit during the entire polymerization process. Interestingly, while *Listeria* and *Shigella* use Arp2/3, the intracellular pathogens *Rickettsia* early in infection use Arp2/3 and later on switch to a different mechanism, expressing proteins that mimic formins to promote actin-based motility (Welch et al., 1998; Suzuki et al., 1998; Gouin et al., 2004; Reed et al., 2014) (see below and Figure 1).

It is however the first time that bacterial mimics of Ena/VASP proteins are characterized (Benanti et al., 2015). The proteins encoded by the two *Burkholderia* species are called BpBimA and BmBimA and display three or one WH2 domains respectively. WH2 domains bind to actin and are present in a number of actin-binding proteins including Ena/VASP proteins. Two hallmarks of Ena/VASP proteins are their properties to oligomerize and to uncap actin filament barbed ends capped by capping proteins. Ena/VASP proteins can thus nucleate actin, elongate filaments at their barbed ends and bundle them.

Previously, it was known that the non-pathogenic species *Burkholderia thailandensis* expressed a BimA protein called BtBimA and that Arp2/3 was necessary

to induce actin-based motility. Here the authors demonstrate that BtBimA has a VCA domain, known to bind and activate Arp2/3. In contrast, this domain is absent in BpBimA or BmBimA of the pathogenic species. A VCA domain is also present in WASP family proteins and in ActA of *Listeria* (Kocks et al., 1992). ActA is a *bona fide* mimics of WASP family proteins (Welch et al., 1998) (Figure 1). In the case of *Shigella*, the outer membrane protein IcsA/VirG (Bernardini et al., 1989) recruits N-WASP (Suzuki et al., 1998) which in turn recruits and activates Arp2/3 (Figure 1). In *Rickettsia*, early during infection, the protein RickA which displays a VCA domain, mimics WASP family proteins and recruits Arp2/3 (Gouin et al., 2004; Reed et al., 2014). Later the surface protein Sca2 acts as a formin (Reed et al., 2014).

BimA proteins as Sca2 belong to a family of bacterial proteins called 'autotransporters' because they have the capacity to insert themselves in the outer membrane, and then display their N-terminal parts on the outside of the bacterium. To this end, they trimerize via a coiled-coil domain which generates a pore through which transport does occur. In the case of BimA proteins, trimerization results in the exposure on the surface of nine WH2 domains for *B. pseudomallei* and three WH2 for *B. mallei*.

In their study, Benanti *et al.* perform a series of *in vitro* assays with purified proteins, mutants analysis, observations of comet tails and plaque assays to analyze the dissemination of the bacteria from one infected cell to its neighbors. They find that BtBimA polymerizes actin if Arp2/3 is present but does not in its absence. In contrast, the two other BimAs are able to polymerize actin in absence of Arp2/3. They also demonstrate that the two proteins remain associated with the barbed ends as the filaments grow. The affinities of BpBimA and BmBimA for the barbed ends are in a similar range to those of formin and Ena/VASP for barbed ends, supporting the idea of they are functionally related. In addition, BimA can gather two filaments and elongate them just as does VASP as well as displace CapZ from the barbed ends. By creating mutations in the coiled-coil domain, they establish that trimerization is critical for actin nucleation, barbed end elongation and anti-capping activity. The authors then compare the tails produced by *B. thailandensis*, *B. pseudomallei* and *B. mallei* and find that they are curved for *B. thailandensis* and straight for the two other species. Importantly, the efficiency of movement (length of displacement) is higher in the case of *B. pseudomallei* and *B. mallei* as these bacteria moved in a linear path in contrast to *B. thailandensis* which moved in curved path, with potential implications for the pathogenicity of these species.

In summary, this study unveils how bacteria can exploit different mechanisms offered by the host cell to polymerize actin. In fact, the results raise the possibility that other different bacteria such as *Rickettsia* species, although closely related, may also use different mechanisms to move inside of cells. Finally, it remains possible that BimA proteins play other roles in infection, as is the case of ActA, that covers the bacterial surface and as a trojan horse protects *Listeria* from autophagy (Yoshikawa et al., 2009),

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### Figure 1. Bacterial actin-based motilities

(A) Schematic representation of the actin-based motilities of *Listeria*, *Shigella*, *Rickettsia spp* and *Burkholderia spp*. (B) Electron micrographs of *L. monocytogenes* tails (left) and *R. conorii* tails (from Gouin et al, 1999 and our unpublished data).